

Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY
RATIONAL MUTAGENESIS

Applicant: VEGA et al.

Filed: December 17, 2001

Examiner: Unassigned.

Our Docket No.: 37851-0911

Appl. No.: 10/022,249

Art Unit: 1643

1st Round: screening of mutants (full length Ala-scan)

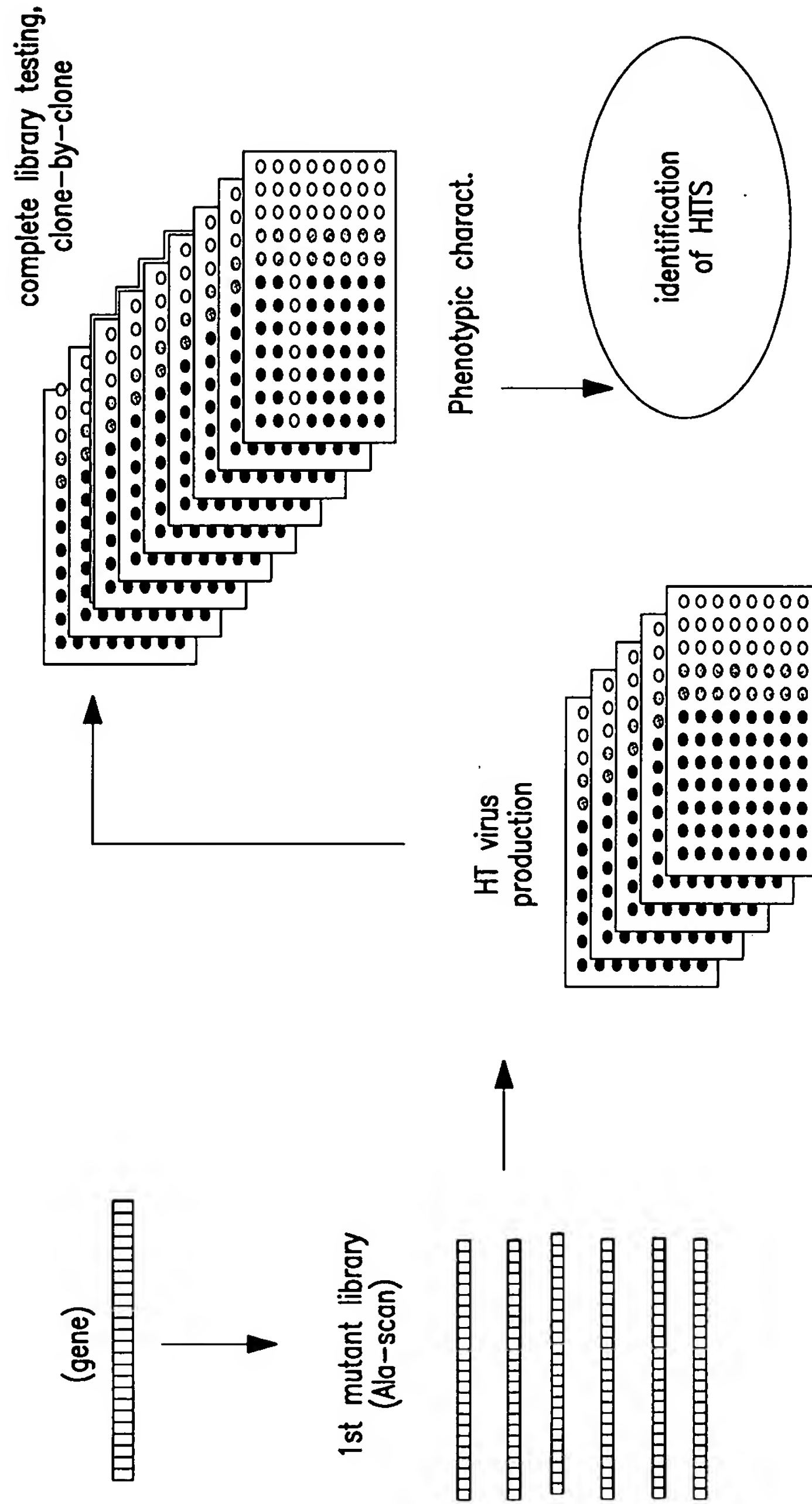


FIG. 1A

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2nd Round: screening of mutants at (surrounding) HIT positions

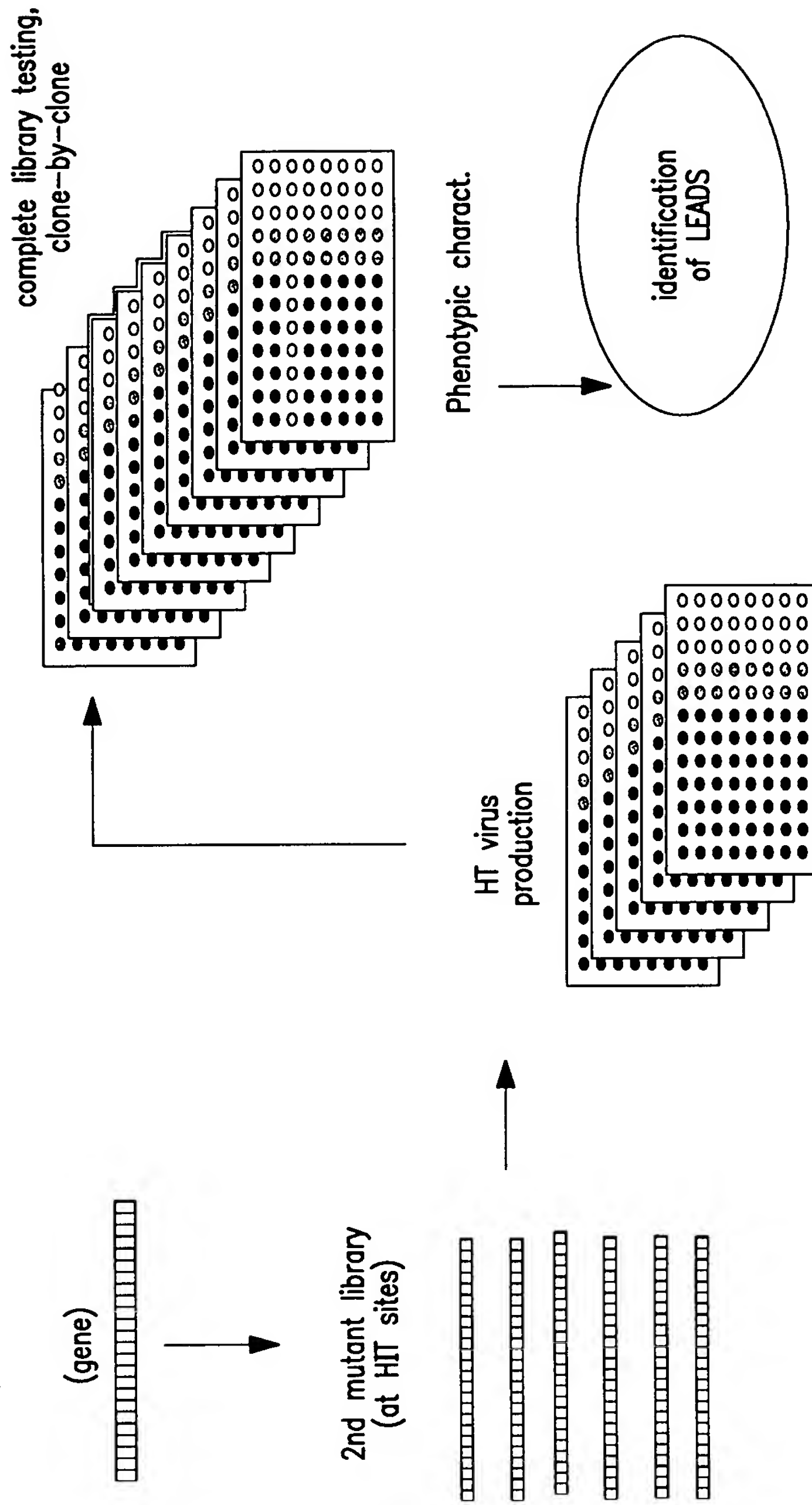


FIG. 1B

3rd Round: screening of recombinants between LEADS

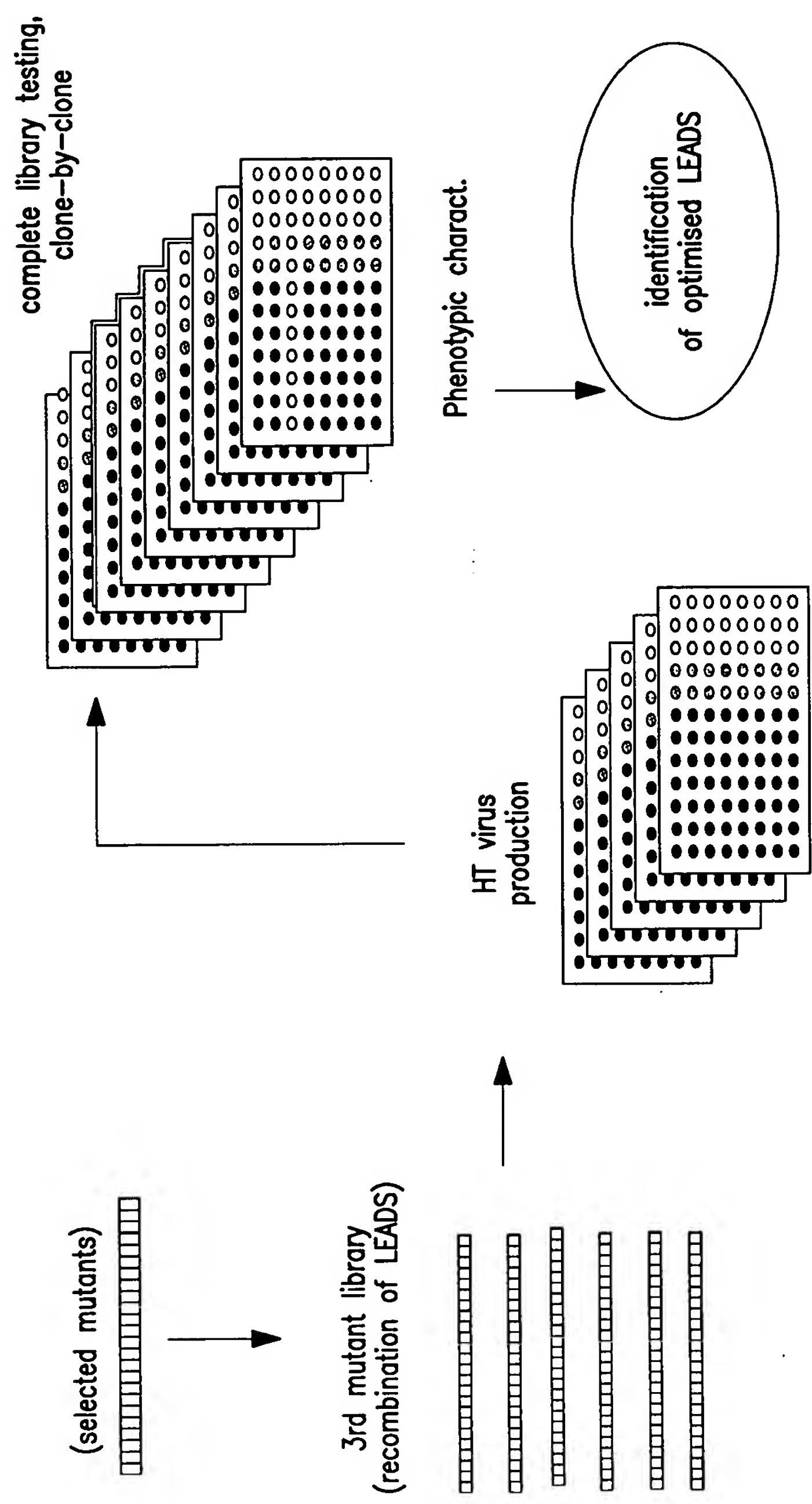


FIG. 1C

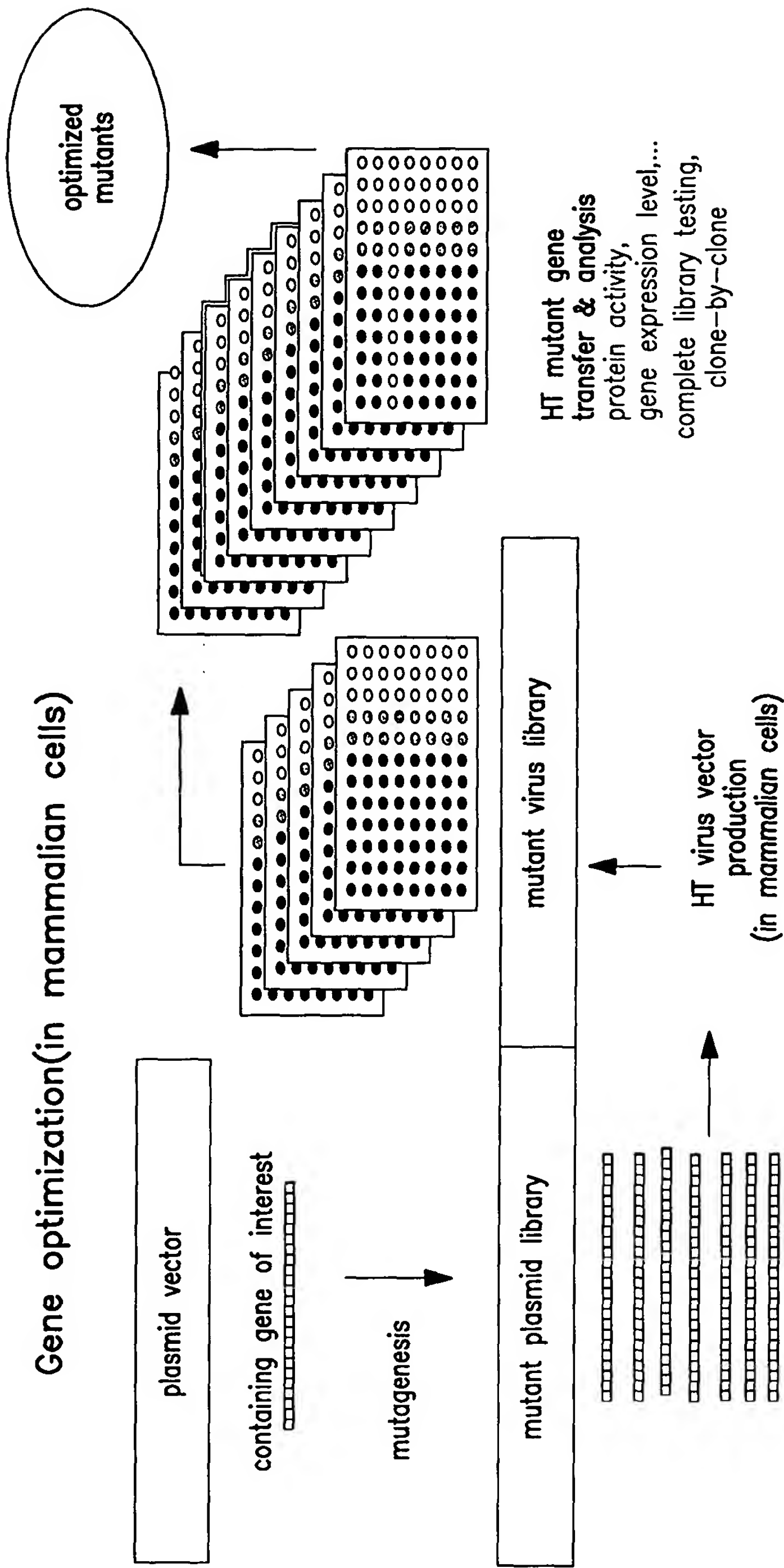


FIG. ID

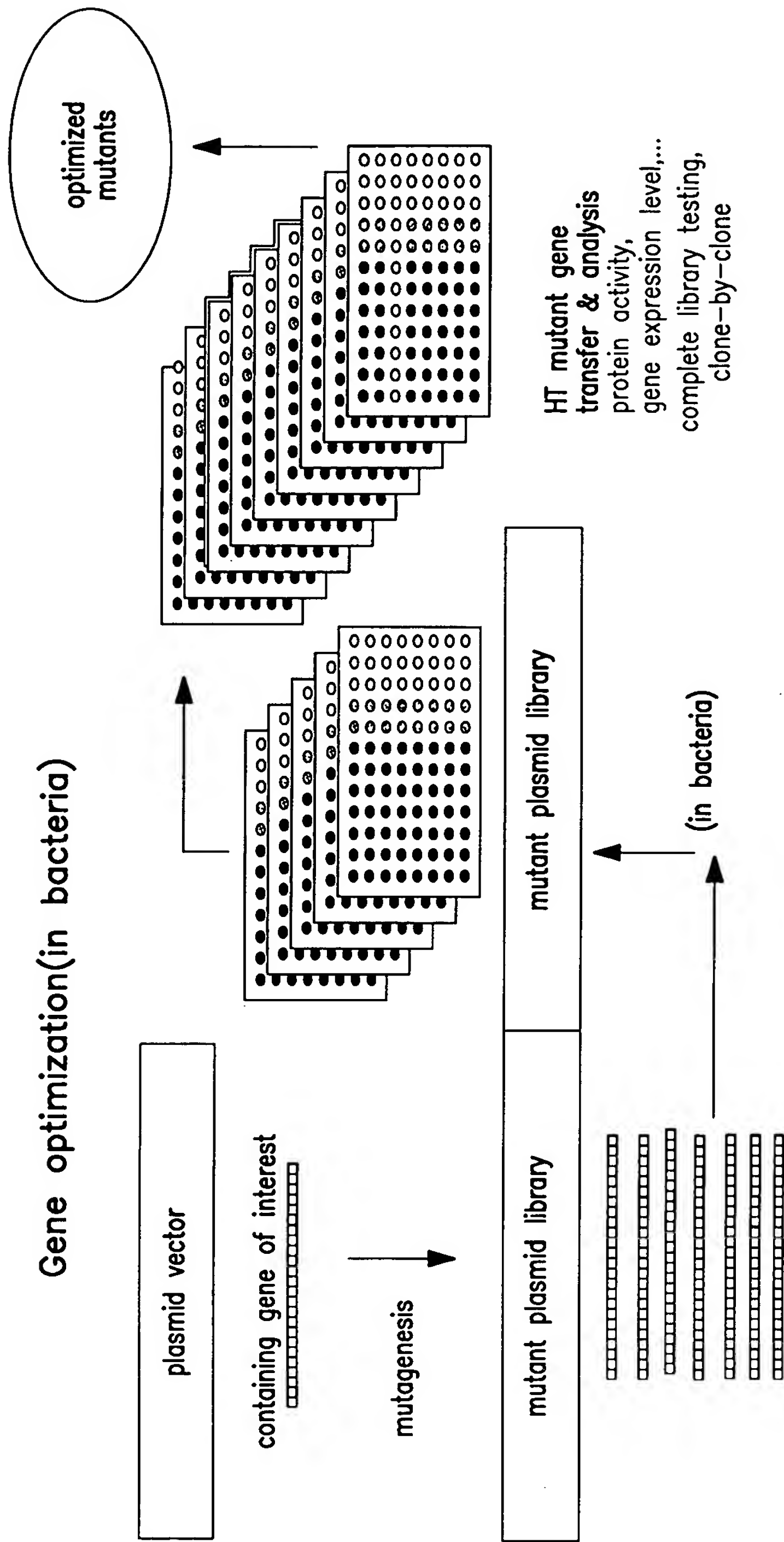


FIG. 1E

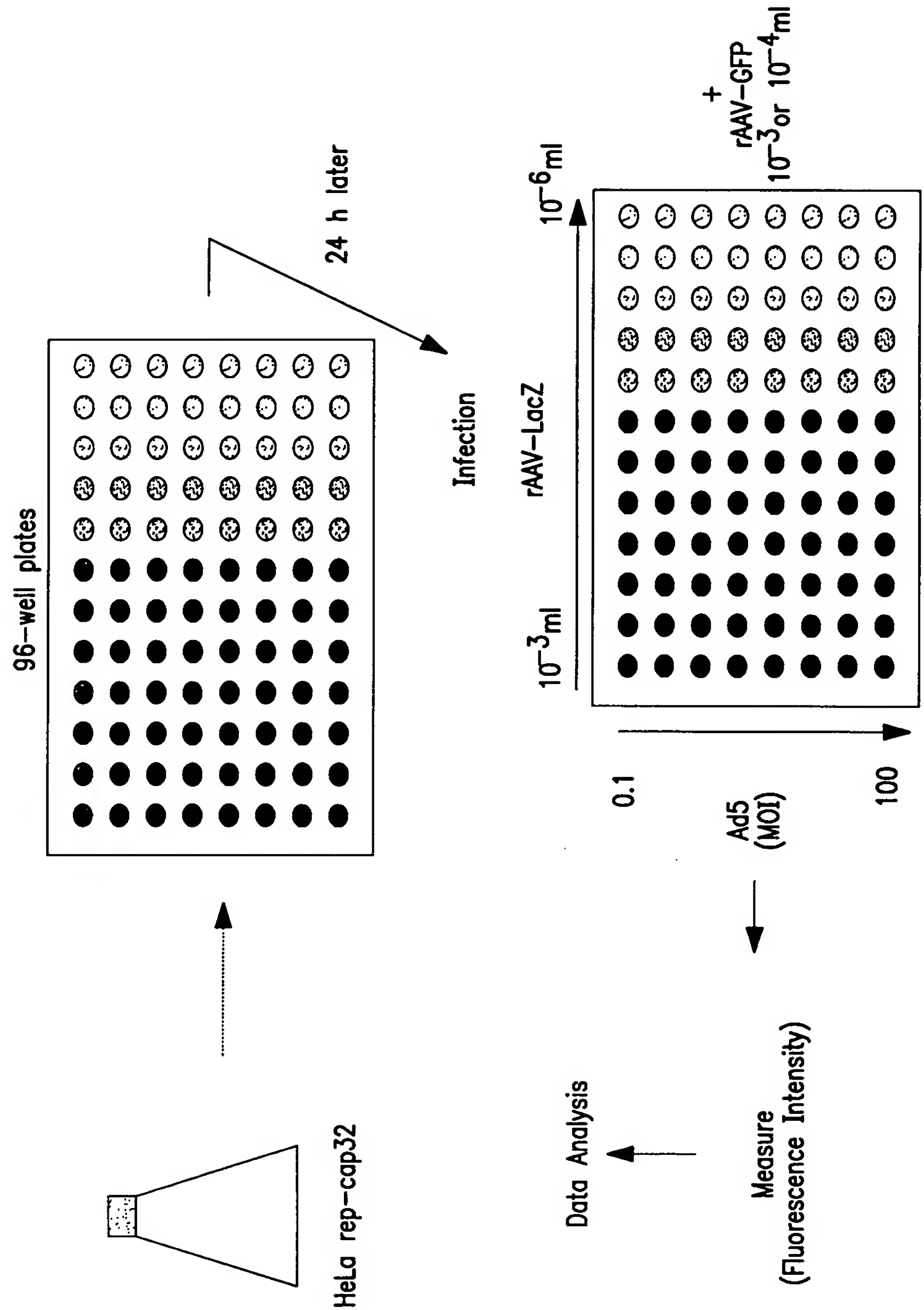


FIG. 2A

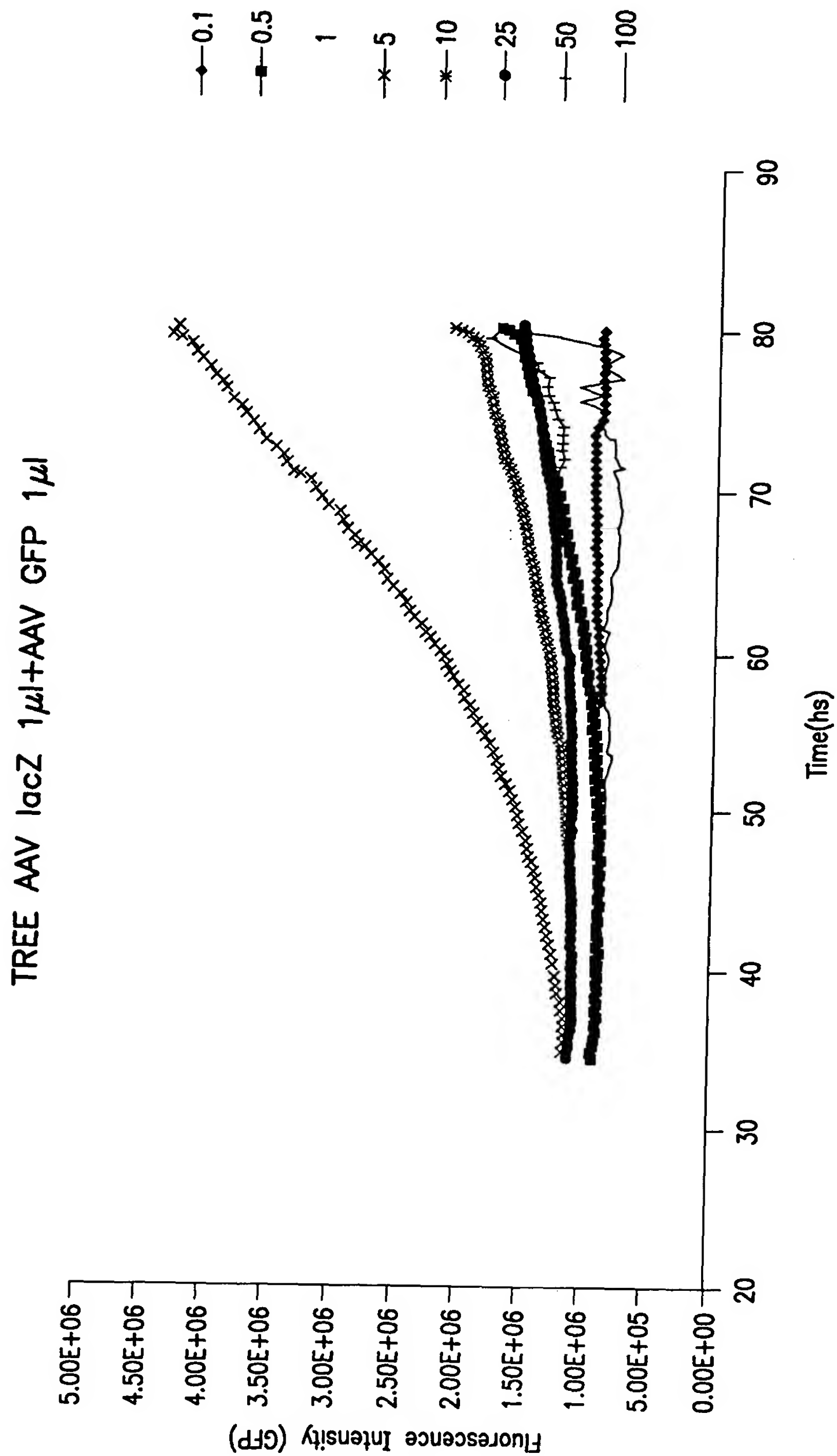


FIG. 2B

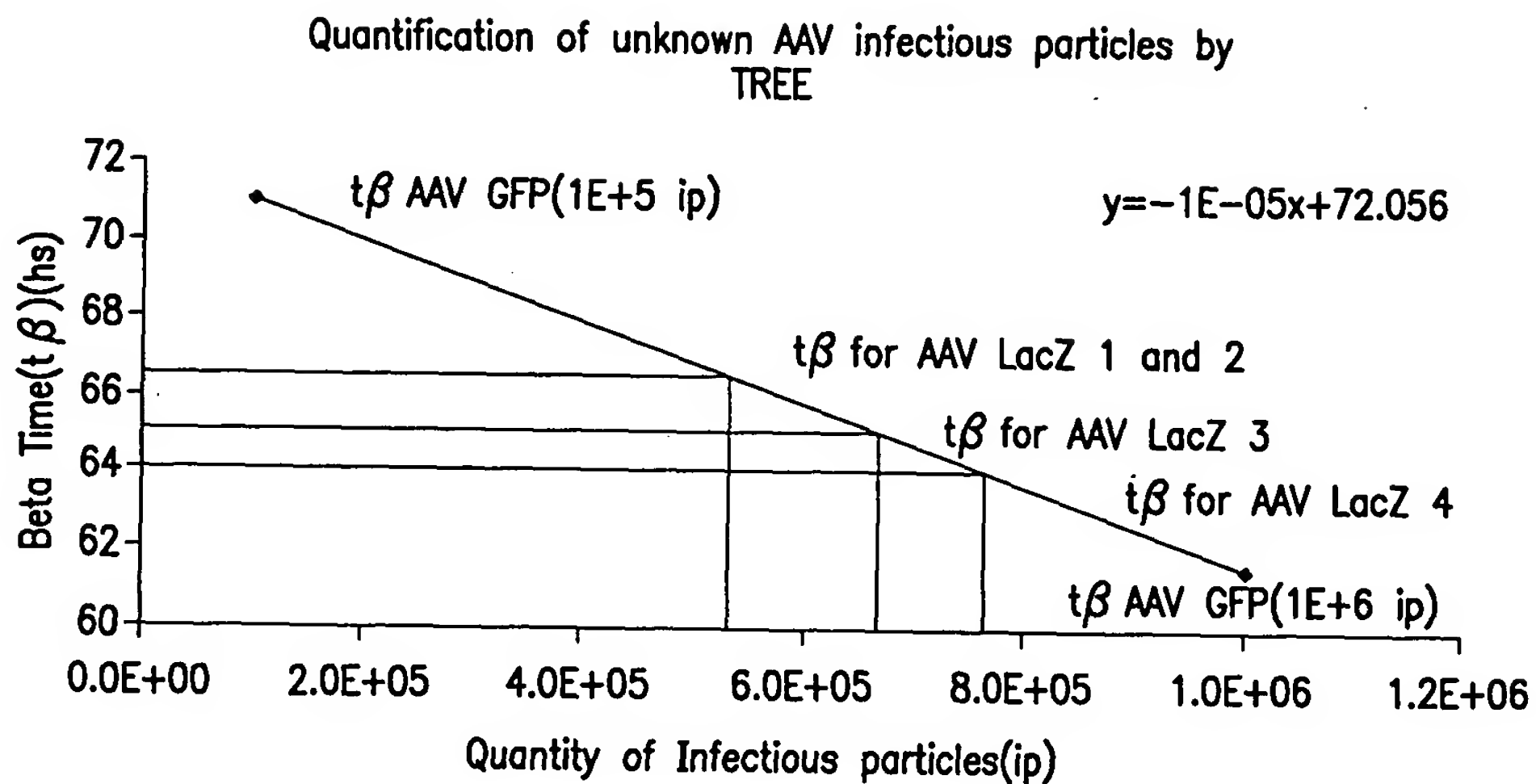


FIG. 2C

Hits on Rep

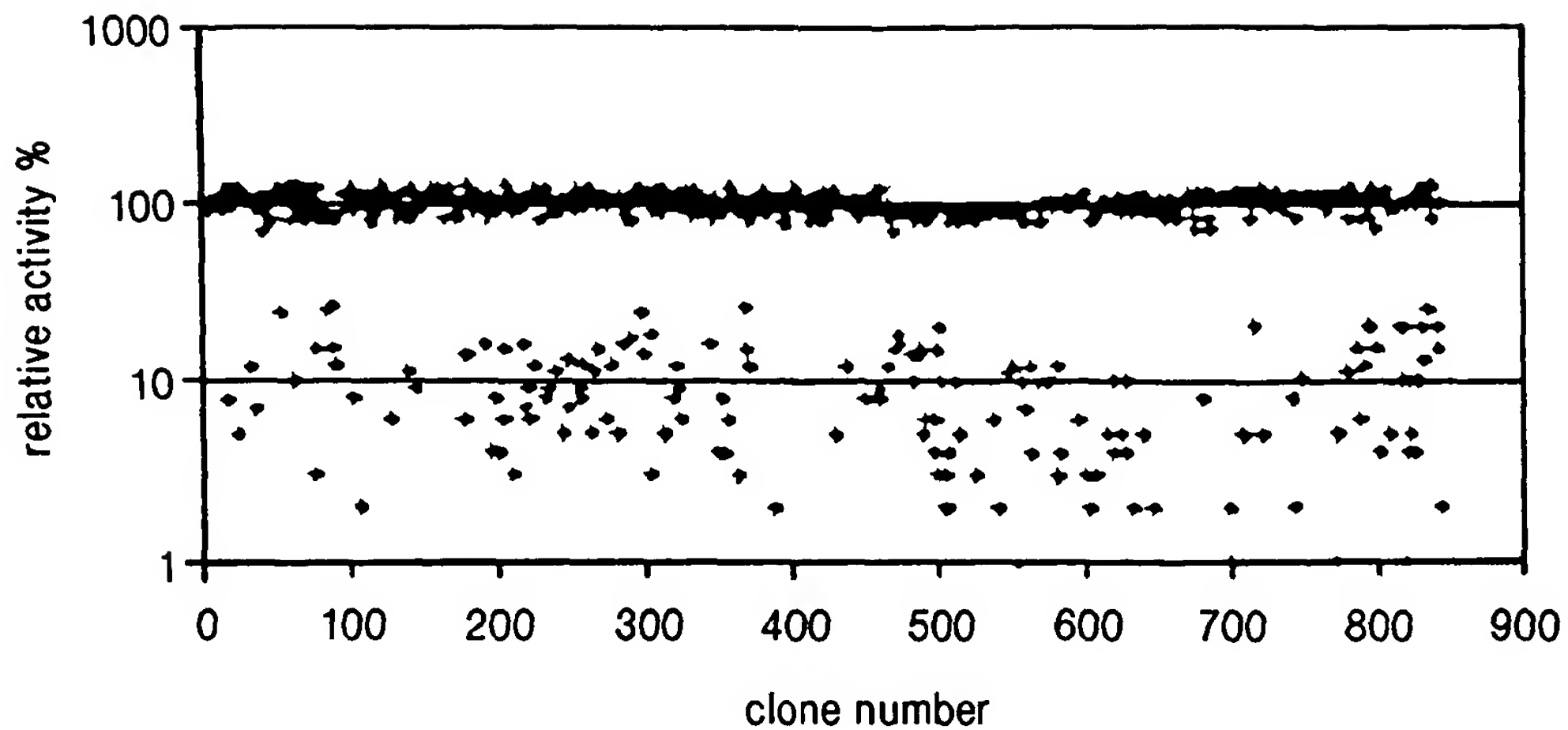


FIG. 3A

Lead positions on Rep

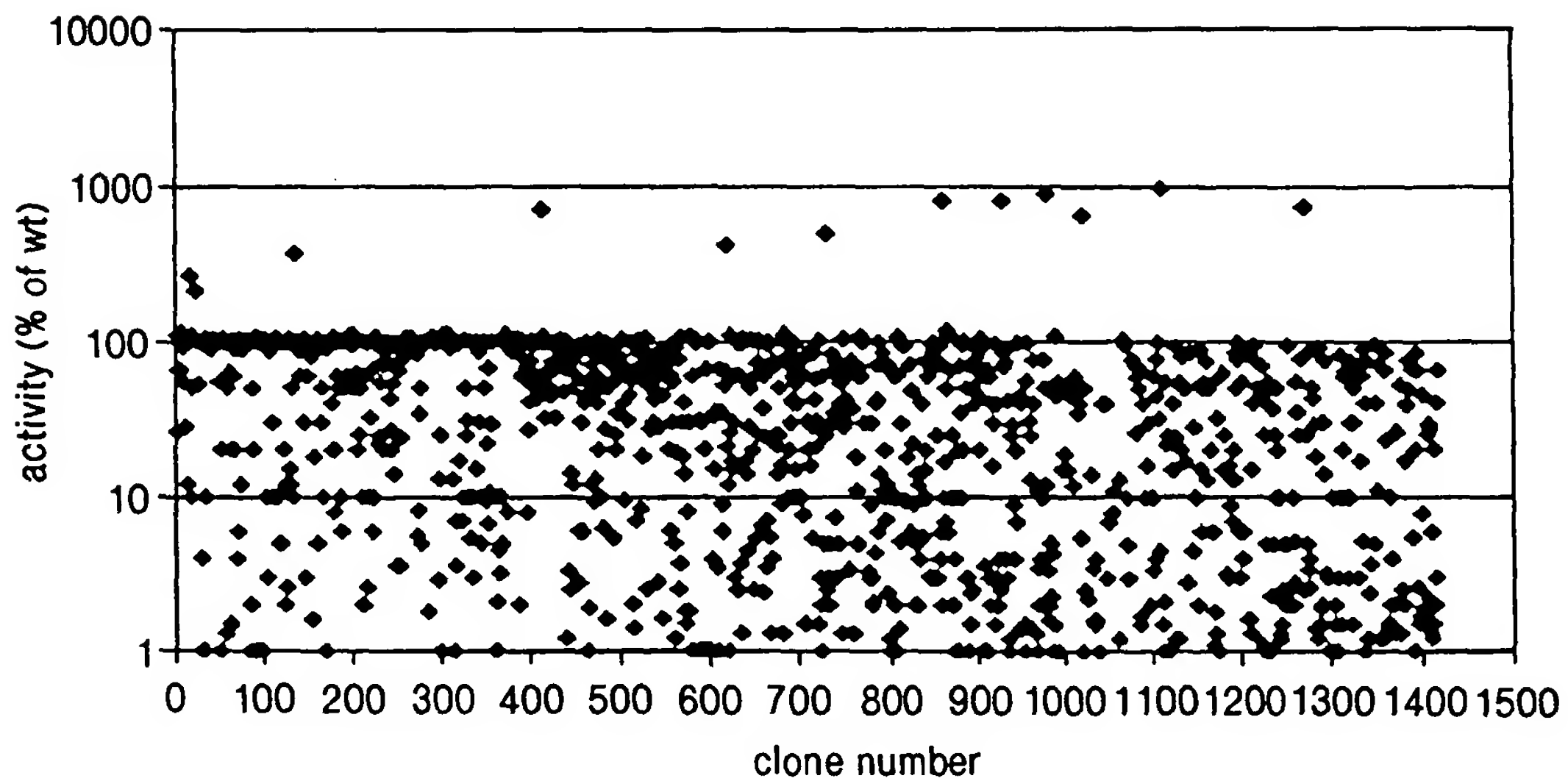


FIG. 3B

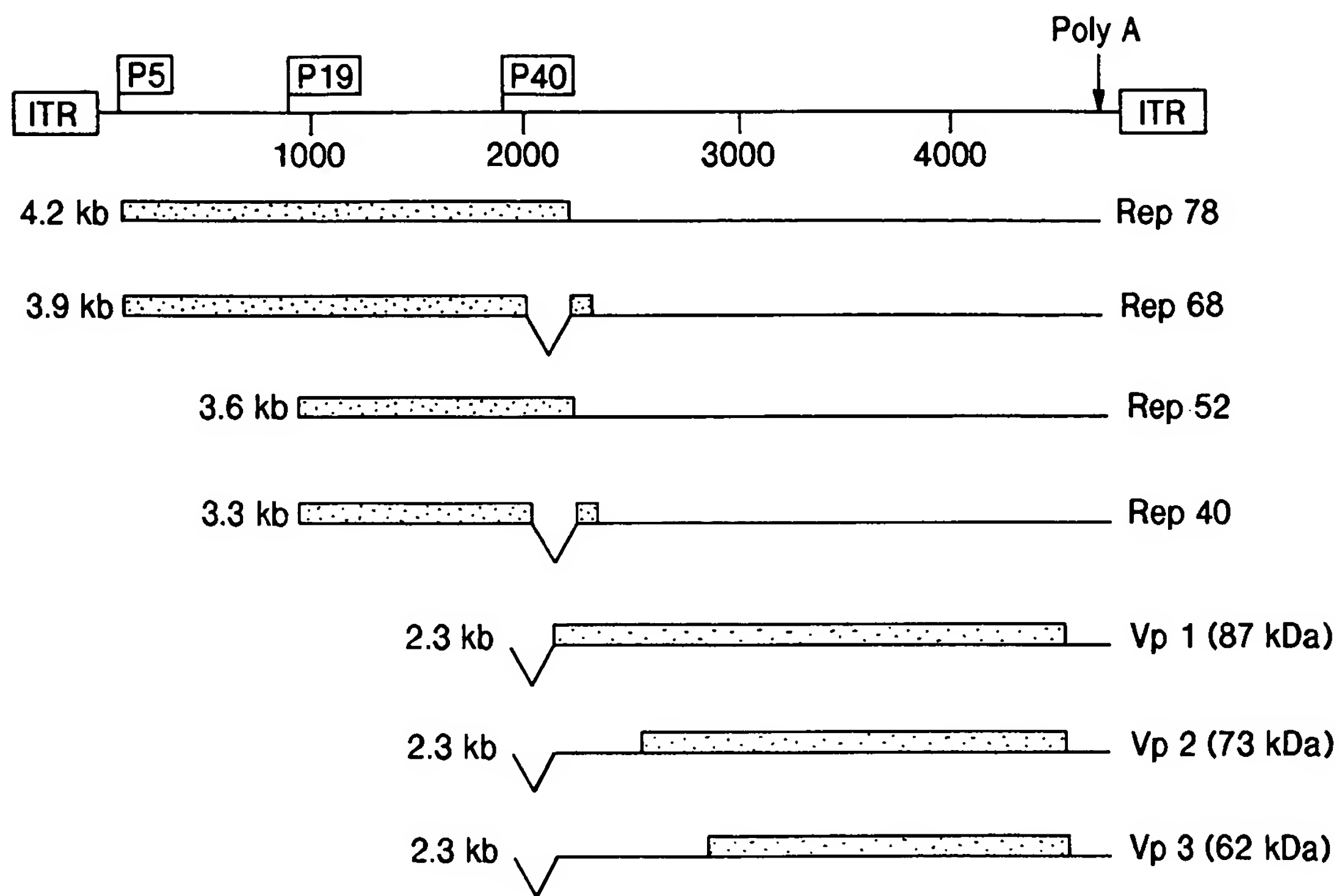


FIG. 4

	10	20	30	40	50	60	
1	MPGFYEIVIKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLI	EQAPLTVAEKLQ	60				
2	MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLI	EQAPLTVAEKLQ	60				
3	MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLI	EQAPLTVAEKLQ	60				
4	MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLI	EQAPLTVAEKLQ	60				
5	MPGFYEIVLKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLI	EQAPLTVAEKLQ	60				
6	MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLI	EQAPLTVAEKLQ	60				
7	MATFYEIVIVRVPFDVEEHLPGISDSFVDWVTGQIWELPPESDLNLT	LVEQPQLTVADRIR	60				
C	M**FYE** : *VP*D***HLPGIS+SFV:WV***WELPP*SD**+*L*EQ**LTVA****						
	70	80	90	100	110	120	
1	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLS	QIRDKLVQTI	120				
2	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLS	QIRDKLVQTI	120				
3	REFLVEWRRVSKAPEALFFVQFEKGETYFHLHVLITIGVKSMVVGRYVS	QIKEKLVTRI	120				
4	REFLVEWRRVSKAPEALFFVQFEKGETYFHLHVLITIGVKSMVVGRYVS	QIKEKLVTRI	120				
5	REFLVEWRRVSKAPEALFFVQFEKGDSYFHLHILVETVGVKSMVVGRYVS	QIKEKLVTRI	120				
6	RDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVKSMVLGRFLS	QIREKLIQRI	120				
7	RVFLYEWNKFSKQ-ESKFFVQFEKGSEYFHLHTLVETSGISSMVLGRYVS	QIRAQLVKVV	119				
C	R:FL++W***SK**E**FFVQFEKG+:YFH*H:L+ET:G**SMV:GR::SQI::*L*::*						
	130	140	150	160	170	180	
1	YRGIEPTLPNWFAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN	MEEYISACL	180				
2	YRGIEPTLPNWFAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN	MEEYISACL	180				
3	YRGVEPQLPNWFAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTN	MDOYLSACL	180				
4	YRGVEPQLPNWFAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTN	MDOYLSACL	180				
5	YRGVEPQLPNWFAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTN	MDOYISACL	180				
6	YRGIEPTLPNWFAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN	MEQYLSACL	180				
7	FQGIPEQINDWVAITKVKK--GGANKVDSGYIPAYLLPKVQPELQWAWTN	LDEYKLAAL	177				
C	**G:EP:***W*A*TK*****GG*NKVVD:*YIP*YLLPK*QPELQWAWTN*::Y:*A*L						
	190	200	210	220	230	240	
1	NLAERKRLVAQHLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWL	VDRGITSEK	240				
2	NLAERKRLVAHDLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWL	VDRGITSEK	240				
3	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWL	VDRGITSEK	240				
4	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWL	VDRGITSEK	240				
5	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWL	VDRGITSEK	240				
6	NLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWL	VDKGITSEK	240				
7	NLEERKRLVAQFLAESSQRS-QEAAASQREFSADPVIKSKTSQKYMALVNWL	VEHGITSEK	236				
C	NL+ERKRLVA*+L***SQ***Q*****+***S**PVI*SKTS**YM*LV*WLV*+GITSEK						
	250	260	270	280	290	300	
1	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVG	PAPPADIKTNRIYR	300				
2	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVG	PAPPADIKTNRIYR	300				
3	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVG	SNPPEDITKNRIYQ	300				
4	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVG	SNPPEDITKNRIYQ	300				
5	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVG	QNPPEDISSNRIYR	300				
6	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVG	QQPVEDISSNRIYK	300				
7	QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVG	SSVPEDISKNRIWQ	296				
C	QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI::NRI*:						
	310	320	330	340	350	360	
1	I LELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAH	AVPFYGCVNWT	360				
2	I LELNGYDPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAH	AVPFYGCVNWT	360				
3	I LELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAH	AVPFYGCVNWT	360				
4	I LELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAH	AVPFYGCVNWT	360				
5	I LEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAH	AVPFYGCVNWT	360				
6	I LELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAH	TVPFYGCVNWT	360				
7	I FEMNGYDPAYAGSILYGWCQRSFNKRNTVWLYGPATTGKTNIAEAIAH	TVPFYGCVNWT	356				
C	I *E+NGY*P:YA:S***GW***:F*KRNT*WL*GPATTGKTNIAEAIAH+VPFYGCVNWT						

FIG. 5A

[illegible]

FIG. 5B